

Integration of single-cell transcriptomic and chromatin accessibility on heterogenicity of human peripheral blood mononuclear cells utilizing microwellbased single-cell partitioning technology

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Poster B902

Abstract

Single-cell RNA sequencing (scRNA-Seq) has significantly advanced our understanding of cell development and heterogeneity in complex systems at cellular resolution. However, its capacity to elucidate cell states and uncover gene regulatory programs remains limited. In contrast, chromatin state profiles serve as a valuable resource for gauging gene expression potential and provide insights regarding transcriptional regulation. When integrated with gene expression data, chromatin accessibility region (CAR) profiles offer a means to define the fundamental gene regulatory logic as the foundation of cell fate.

ATAC-Seq (Assay for Transposase-Accessible Chromatin using Sequencing) is a potent approach for profiling genome-wide CARs. To capture both mRNA gene expression data and gene regulatory information in a single assay, we conducted multiomic scATAC-Seq + scRNA-Seq on PBMCs, utilizing the gentle and robust microwell-based single-cell partitioning technology of the BD Rhapsody[™] System. This scATAC-Seq assay showed high sensitivity (many unique fragments of appropriate size per cell) and specificity (most fragments clustered into distinct peaks, with significant enrichment around annotated Transcription Start Sites). Here we demonstrate the power of this multiomics assay for exploring the regulation of gene expression through chromatin accessibility. Cell types were annotated, and we found enrichment of transcription factor motifs within CARs in each distinct cell type. Gene accessibility correlated well with RNA expression within cell types, and we demonstrate that more in-depth examination of gene regions can indicate the presence of enhancer elements.

<u>Results</u>

Sensitivity and specificity metrics for the BD Rhapsody[™] **Single-Cell ATAC-Seq Assay**

Figure 2. Isolated nuclei form human peripheral blood mononuclear cells (PBMCs) were subjected (A) to scATAC-Seq and mRNA Whole Transcriptome Analysis (WTA) Library Preparation using the BD Rhapsody[™] System. (A) Fragment size distribution plot, demonstrating a preferential transposition of the nucleosome-free regions of open chromatin (large peak at <147 bp) and further peaks reflecting the ~147 bp repeating Fragment size (bp) pattern of nucleosome positioning. (B) Fraction of Tn5 transposase sites from each cell within identified chromatin peaks, highlighting precise chromatin targeting. (C) Transcription Start Site (TSS) enrichment plot, displaying the read density around TSSs from the genomic background, emphasizing the accessible state of promoter regions for transcriptional activities. (D) Scatter plot of BD Rhapsody[™] beads showing cell-calling from a multiomic experiment with Distance from TSS (bp) human PBMCs. RNA molecules are plotted transposition events for each bead, and the beads that were called as true cells by RNA, ATAC, and both assays are indicated by color.



Plasmab B memory

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● CD14 Mono ● HSPC

cDC2

CD16 Mono 🗕 Other

CD8 Naive

UMAP 1

CD8 memory • NK_CD56bright • Plasmablast • pDC

B memory

Transcription factor motif enrichment across cell types

											- 1 0
AAASeccaagte	SPIB -	0.8	1	0.6	0.58	0.06	0.05	0	0.08	0.07	- 1.0
AGAGGAAGTG	PU1 -	0.73	1	0.56	0.45	0.09	0.12	0.14	0	0.2	
ACTICCLERE	ELF4 -	0.83	1	0.8	0.71	0.32	0.45	0.56	0	0.51	- 0.8
GRAASIGAAASI	IRF8 -	1	0.91	0.34	0.24	0.12	0.06	0	0.03	0.1	
GGAASTGAAAST	PU1-IRF8 -	1	0.99	0.39	0.27	0.13	0.11	0.08	0	0.22	
ATTGGGCAAC	CEBP -	0.14	0.74	1	0.64	0.18	0.12	0.07	0	0.21	- 0.6
<u><u></u></u>	NFIL3 -	0.08	0.67	1	0.63	0	0.14	0.05	0.06	0.14	
SAACCACAS	RUNX -	0.14	0.43	0.08	0	0.64	0.68	0.76	1	0.97	
<u><u>ACCCGGAAGT</u></u>	GABP -	0.6	0.31	0.36	0.27	0.61	0.75	1	0	0.84	- 0.4
ASGTGISA SE	T-bet -	0.17	0.06	0.03	0	0.84	0.06	0.47	0.12	1	
CITTGAIGTESS	TCF7 -	0.09	0	0.08	0.07	0.11	1	0.9	0.62	0.17	
ACAGGAASTS	ERG -	0.34	0.34	0.2	0	0.39	0.78	1	0.19	0.75	- 0.2
AASTAGGTCA	RORγt -	0	0.13	0.02	0.02	0.04	0	0.01	0.86	1	
<u><u><u></u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u></u>	RORα -	0.01	0.09	0.03	0.04	0.05	0.02	0	0.63	1	
e 5. The heat may ormalized enrichmo ll-type-specific tra	p displays ent scores nscription	- B	Dendritic -	Classical Monocyte	Jonclassical Monocyte	atural Killer -	Naive CD4 -	Naive CD8 -	lemory CD4 -	emory CD8 -	0.0
r motifs <u>A</u> motif	score ic				~	Z			2	\geq	

- 1.0

Methods

Workflow of Single-Cell ATAC-Seq and mRNA Whole Transcriptome Analysis on BD Rhapsody[™] HT Xpress System



RNA and ATAC data can be combined for dimensionality reduction **Figure 3.** Dimensionality reduction is NK_CD56bright NK CD56dim HSPC Uniform Manifold performed bv Approximation and Projection (UMAP), utilizing scRNA and scATAC-Seq data jointly. These cells were then annotated as members of distinct cell types using a PBMC reference atlas (Hao 2021) and the R

Figure the no of cel factor motifs. A motif score is calculated based on binomial distribution, using Homer

For each patr of wa cell type and a transcription factor, it calculates a relative motif enrichment in the differentially accessible regions of the given cell type, relative to randomly-selected background regions with controlling GCcontent. Then the motif scores were normalized across cell types for each motif, with a scale ranging from 0 to 1. A score of 0 represents the least enrichment, while a score of 1 represents the highest enrichment of the motif. Models of transcription factor binding motifs are displayed to the left.

Expression correlates with gene accessibility within cell types

Figure 6. This heatmap represents correlation Pearson's coefficients between scATAC-Seq gene activity scores corresponding gene values aggregated by within a human PBMC c Y-axis corresponds to measured gene expres the X-axis is inferred "ge based on transposase ad and 2000bp upstream o gene bodies. There correlation betwee accessibility and expres most of the cell-types. symmetrical heatmap comparing the ATAC acc one cell group to anot RNA expression is not comparing the RNA fro with the group accessibility of the second that correlation is his perfect: if correlation we there would be no poi both assays.

replicates using standalone and multiomic scATAC-

Other- -0.31 -0.32 -0.03 -0.16 0.4 -0.04 0.31 0.41

Figure 1. Following the isolation of nuclei from cells, they undergo a tagmentation process utilizing a custom Tn5 transposase. This critical step involves the selective cutting of accessible chromatin regions, during which adapter sequences (pre-loaded onto the Tn5) are concurrently attached to both ends of the resulting fragments. The tagmented nuclei are then precisely loaded into a BD Rhapsody[™] 8-Lane Cartridge, where they are individually partitioned into wells equipped with BD Rhapsody[™] Enhanced Cell Capture Beads. This process ensures the simultaneous capture of both ATAC and mRNA fragments on the beads. Subsequently, distinct Whole Transcriptome Amplification (WTA) and ATAC libraries are prepared from these beads. These libraries are then sequenced and processed through a comprehensive analysis pipeline, leading to the generation of highquality data that is fully compatible with existing bioinformatics tools, ready for in-depth analysis.

Summary

Empowering Molecular Biology and Immunology research with an accessible Multiomic scATAC-Seq Assay and analysis pipeline.

• Our study successfully integrates scRNA-Seq and scATAC-Seq through the BD Rhapsody[™] Single-cell Analysis System for

packages Seurat v4.4 and Signac v1.12. These annotations are based on the RNA data, but as high-quality reference atlases become more common, the same approach will be applicable to scATAC-Seq data. Lower-resolution annotations are already

possible by inferring gene activity from gene body accessibility (See Figure 5).

()

Peak



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CD4 Naive on dnT

🕨 CD4 memory 🔍 MAI

gene activity scores and the	Dendritic -	-0.47	-0.47	-0.15	-0.36	0.46	0.28	0.59	0.4				
values aggregated by cell type													
within a human PBMC data set. The	D		0.4	0.26	0.26	0 00	0.02	0.26	0.02				
Y-axis corresponds to direct RNA-	UO B-	-0.37	-0.4	-0.20	-0.30	0.08	0.82	0.20	0.02				
measured gene expression, while	SSI												
the X-axis is inferred "gene activity",	Monocyte -	-0.54	-0.53	-0.13	-0.36	0.69	0.03	0.45	0.61				
based on transposase activity within	ex												
and 2000bp upstream of annotated	U DO	0 1 0	0.27	0.26	0 69	0.25	0 22	0 27	0 1 0				
gene bodies. There is high	0 MAI1 -	0.10	0.27	0.20	0.00	-0.25	-0.52	-0.27	-0.10				
correlation between gene	AN												
accessibility and expression within	🗠 Natural Killer -	-0.19	0.15	0.78	0.29	-0.07	-0.24	-0.09	-0.03				
most of the cell-types. This is not a													
symmetrical heatmap because		0.51	0 7 2	0 16	0.26	0.46	0 10	0.45	0 20				
comparing the ATAC accessibility of	10	0.51	0.75	0.10	0.50	-0.40	-0.40	-0.45	-0.50				
one cell group to another group's	110												
RNA expression is not the same as	0.5 CD4 T-	0.76	0.47	-0.26	0.2	-0.43	-0.44	-0.41	-0.38				
comparing the KNA from the first	0.0												
accessibility of the second It is good	0.5	~	- ~	, e ^t	- 	, NO	\diamond	, tic	, et				
that correlation is high but not	-0.5	CDr	00	al till	When	noch		andrit	O _{fl}				
nerfect: if correlation were perfect	-1.0		Natur	•	h	\diamond							
there would be no point in doing	Conelation	Correlation \sim ATAC gene activity											
both assays.	oth assavs.												
High reproducibility across PBMC replicates with BD Rhapsody 11													
multiomic ccATAC Son accourse A noaly comparison analysis													
multionic scarac-seq assays: A peak comparison analysis													
Figure 7. (A) Correlation analysis of F	PBMC sample (A)					(B)							
		4											

comprehensive multiomic analysis of PBMCs from multiple donors. • This gentle approach allows for the simultaneous examination of gene expression and chromatin accessibility, providing a deeper understanding of cellular heterogeneity, development, and gene regulatory mechanisms.

• Our analysis reveals enriched transcription factor motifs and demonstrate a correlation between mRNA profiles and chromatin accessibility regions (CARs).

• Our data demonstrate consistency across both standalone scATAC-Seq and multiomic scATAC-Seq + scRNA-Seq analyses.

• Results of our pipeline are formatted for immediate compatibility with commonly used bioinformatic tools, allowing a quick and easy start to downstream analysis.

55900000 55950000 56000000 55850000 chr8 position (bp)

Figure 4. Aggregate ATAC signal within each cell type in the region surrounding the differentially expressed gene LYN. Log-normalized expression of LYN in the cells of each group is displayed in violin plots. Detected peaks of transposase activity are shown below the gene annotation. Note that some obvious peaks are visible upstream of the gene TSS, indicating a likely enhancer element (dotted box).

pearsonr: 0.96 spearmanr: 0.93 pearsonr: 0.95 spearmanr: 0.92 Seq assays demonstrates strong agreement between techniques through peak comparison. The analysis reveals a high correlation, indicating that the WTA assay does not interfere with the ATAC results. (B) There is also high correlation in the ATAC peaks found between two experiments performed by different scientists on the same PBMC sample, underscoring the reproducibility and reliability of the technique across multiple experiments. BD Rhapsody[™] scATAC-Seq BD Rhapsody[™] Multiomic scATAC-Seg Experimenter 2

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